Age-related macular degeneration (AMD), the major cause of blindness in adults (65 years of age and older), and diabetic retinopathy, the major cause of blindness in working adults, are chronic, progressive diseases with multifaceted etiologies that are not fully understood. Progression and lack of treatment of both diseases may lead to the advanced stage with neovascularization. Although the detailed cellular mechanisms leading to the development of AMD and diabetic retinopathy remain elusive, oxidative damage to the retina and its pigment epithelium are considered to be involved. Clinical studies have shown that the progression of AMD can be slowed down by nutritional antioxidants, but trials with antioxidants for diabetic retinopathy (very limited in number) have been inconclusive. Long-term administration of the AREDS antioxidants, the same nutritional antioxidants that have been demonstrated to slow the progression of AMD, have yielded exciting results in preventing the pathogenesis of retinopathy in diabetic rodents. These results suggest the merit of testing the AREDS antioxidants in a clinical trial to prevent the development and/or progression of diabetic retinopathy, with the possibility of reducing the impact of this common vision-threatening disease. (Invest Ophthalmol Vis Sci. 2011;52:8665–8671) DOI: 10.1167/iovs.10-6768

More than 6.5 million Americans older than 65 years have severe vision impairment, and as the population ages, the number is expected to double by 2030. Vision impairment has a direct impact on the quality of life and on the independence of an individual. The two major chronic eye diseases associated with vision loss are age-related macular degeneration (AMD) and diabetic retinopathy. Rates of depression have reached 20% in patients with AMD, even after the breakthrough treatment with anti-VEGF. Older patients with newly diagnosed AMD have higher rates of depression and hip fracture, than those without AMD. Individuals with AMD also have a higher prevalence of 11 of 16 general health conditions than do controls, and this results in a major impact on resource commitment. Diabetic retinopathy is the leading cause of blindness in young adults. This microvascular complication is also closely associated with a greater risk of other vascular complications, such as stroke, coronary heart disease, and heart failure. Although AMD and diabetic retinopathy stem from different causes, they both can target the vasculature (AMD-choroidal neovascularization, and diabetic retinopathy-retinal neovascularization), and their multifaceted etiologies share many common features.

**Age-Related Macular Degeneration**

Age-related macular degeneration is the leading cause of vision loss in the United States in patients 65 years of age or older. Current estimates predict that approximately 10% of the population in the 66- to 74-year age group has some form of macular degeneration, and this increases to 30% in the 75- to 85-year age group. More than 54% of all blindness (~1.75 million) in adults 40 years of age and older in the USA is attributable to AMD. These numbers are expected to reach up to 3 million by 2020. The disease results in damage to various layers of the retina, including retinal pigment epithelium (RPE), Bruch’s membrane, the choroid, and outer retina. AMD is divided into two major clinical forms, dry and wet AMD. In the dry form, which accounts for more than 85% of the cases, with aging and thinning of the macular tissue and atrophy of the RPE and adjacent cells in contiguous areas of the macula, subretinal deposits (drusen, an insoluble material) start to accumulate between the RPE and the underlying choroid. The wet form of AMD, which accounts for approximately 15% of patients, is characterized by choroidal neovascularization. Although wet AMD is less common than dry AMD, it is usually more aggressive and can cause rapid and severe vision loss. In some cases, dry AMD can also progress into wet AMD. VEGF is secreted by the RPE at its basal side and helps maintain the choriocapillaries. The thickening of Bruch’s membrane in aging impairs the diffusion of VEGF and results in hypoxia. Hypoxic conditions further increase VEGF, and choriocapillaries start to undergo neovascularization. AMD is also associated with some genetic and environmental factors; and, although there is no clear genetic marker, the first-degree relatives of patients with AMD are at a higher risk of developing the disease. In addition, cigarette smoking, high blood pressure, exposure to sunlight, and a diet rich in linoleic acid and monounsaturated, polyunsaturated, and vegetable fats are also associated with AMD.

**Molecular Mechanisms of AMD**

The retina is vulnerable to oxidative damage; RPE cells are especially exposed to high oxidative stress. With aging, antioxidants decrease, the capacity to degrade damaged protein and lipid is weakened, and the ability to repair damaged DNA is reduced. Because of a decline in physiological function or gradual loss of RPE, phagocytosis of the photoreceptor is compromised, and lipofuscin (damaged proteins and lipids) accumulates in the RPE. Oxidative damage to the retina manifests in the loss of retinal cells, formation of drusen, and accumulation of degradation products in Bruch’s membrane.

Mitochondrial impairment and genomic instability also contribute in age-related changes and pathology. The number and area of mitochondria in the RPE are decreased, and their DNA is damaged. The haplogroups in mtDNA associated
with AMD are altered, the frequency of mtDNA SNPs associated with the haplogroups J, T, and U is increased, and trafficking and protein folding are also compromised.

Genetic studies have shown a strong association between variations in the complement factor H gene, a gene that affects inflammation and dysfunction of the immune system, and the risk of developing AMD. Almost 45% of patients with AMD have a genetic mutation in the complement factor H gene. Macrophages and leukocytes start to appear near drusen and neovascular membrane, and macrophages not only produce reactive oxygen species (ROS) by phagocytosis, they also stimulate VEGF and secrete cytokines. Increased VEGF further promotes neovascularization in the choroid, forming fragile new vessels, which begin to bleed, with loss of photoreceptors. Despite extensive research in elucidating the possible mechanisms, the exact pathophysiology remains unclear. To make a bad situation worse, the search to identify specific mechanisms is also hindered by the lack of an in vitro system for dry AMD and of a proper animal model, because of the absence of a well-developed macula in nonprimates.

### Diabetic Retinopathy

Diabetes has become the epidemic of the 21st century. The total number of people with diabetes worldwide was 171 million in 2000, and the number could rise to 366 million by 2030. Diabetes results in many overlapping and interrelated pathways that are responsible for both initial vascular insult leading to retinopathy and the continued tissue insult leading to macular edema. Diabetic retinopathy is characterized by gradual and progressive alterations in the retinal microvasculature. Although hyperglycemia is considered to be the major initiator of diabetic retinopathy, hypertension, and hyperlipidemia are also closely associated with its progression. Despite extensive research in the field, the exact etiology of this multifactorial disease remains elusive.

Another cause of vision impairment faced by diabetic patients is macular edema; the risk of developing macular edema is closely associated with the degree of diabetic retinopathy. Because of breakdown of the blood-retinal barrier, an early hallmark of diabetic retinopathy, fluid and protein collect on or under the macula, causing it to thicken and swell. As with AMD and diabetic retinopathy, macular edema is also a progressive disease that can result in visual loss. It is crucial to detect the early signs of retinopathy in a diabetic patient to prevent further progression.

### Molecular Mechanisms of Diabetic Retinopathy

The pathogenesis of diabetic retinopathy is complex and involves several molecular and biochemical mechanisms that affect cellular metabolism in the retina. Extensive research in the area has implicated various pathways in its development, including oxidative stress, inflammation, the polyl pathway, accumulation of advanced glycation end-products (AGEs), and protein kinase C (PKC) activation. Although the pathology is manifested in the microvascular cells, many other cells including Müller and glial cells are also compromised and their role in the development of diabetic retinopathy has become the focus of research in many leading laboratories.

Results have shown that mitochondrial overproduction of superoxide, by regulating glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and increasing diacylglycerol levels, can act as a unifying mechanism regulating the major metabolic pathways implicated in the development of diabetic retinopathy. Increased formation of diacylglycerol in diabetes leads to the activation of PKC, which, in turn phosphorylates and thus activates NADPH oxidase, increasing the production of ROS, and further activating the polyl pathway and AGEs formation. Activation of metabolic pathways and their downstream effectors in the diabetic milieu can also increase oxidative stress, suggesting a bidirectional mechanism. Mitochondria are dysfunctional, the electron transport system becomes subnormal, and mtDNA is damaged, fueling a vicious cycle of sustained superoxide accumulation. Causing further exacerbation, the activity of complex III of the electron transport chain is impaired, and the antioxidant defense system to combat increased oxidative stress is also compromised. Thus, increased oxidative stress and dysfunctional mitochondria with its DNA damaged initiates a vicious cycle of increased superoxide accumulation, which further fuels the continued progression of diabetic retinopathy. Because of increased superoxide radicals, hydrogen peroxide levels are increased, and increased hydroxyl radical (formed via Fenton reaction) continue to compromise the mitochondria.

The proangiogenic factor VEGF is also under the control of oxidative stress, and activation of the polyl pathway has the potential to further elevate VEGF, possibly via increasing NADH oxidase activity. Furthermore, to exacerbate this bad situation, VEGF itself can also induce formation of superoxide. Increased oxidative stress activates the redox-sensitive nuclear translocation factor NF-kB, and activation of NF-kB modulates the expression of the inducible form of NO, which, in turn, results in increased free radical production. The reaction between superoxide and nitric oxide is accelerated, resulting in increased peroxynitrite levels, and increased peroxynitrite exerts its damaging effects via many pathways, including increasing VEGF levels and accelerating apoptosis.

Animal models have shown a role of poly ADP-ribose polymerase (PARP) in the development of diabetic retinopathy, possibly via regulation of NF-kB. PARP activation also slows down the glycosylation and electron transport via depleting NAD+ and inhibits GAPDH by poly-ADP-ribosylation, and in the pathogenesis of diabetic retinopathy, increased retinal GAPDH is ribosylated and nitrated.

Diabetic retinopathy also shares many characteristics with a low-grade, chronic inflammatory disease. The levels of inflammatory mediators (e.g., interleukin-1β, tumor necrosis factor-α, intercellular cell adhesion molecule, and vascular cell adhesion molecule) are increased in the vitreous and the retina of diabetic patients, and animal models have shown a close association of diabetic retinopathy with increased leukostasis and cytokine levels. The polyol pathway also mediates other hyperglycemia-induced molecular abnormalities in the retinal vessels, including TGF-β overexpression, and thus, the regulation of the TGF-β pathway could be beneficial in ameliorating the development of diabetic retinopathy. Impaired retinal perfusion directly affects the oxygen delivery to the retina, and hypoxia is observed early in the pathogenesis of diabetic retinopathy. Retinal capillaries become nonperfused, and occlusion of the retinal vasculature damages retinal neurons and suppresses leukocyte-endothelial cell interactions. Furthermore, patients with diabetic retinopathy have decreased oscillatory potential amplitude, delayed oscillatory potential latency, and abnormal multifocal ERGs. Thus, the etiology of diabetic retinopathy is complex and affects metabolic, hemodynamic, and functional aspects of the retina.

### Therapies with the Potential to Inhibit AMD and Diabetic Retinopathy

As presented above, both AMD and diabetic retinopathy are slow-progressing ocular diseases that can result in neovascularization. Most treatment options are aimed at stopping the leaking blood vessels and/or preventing neovascularization-
tion. Several antiangiogenic therapies, including VEGF-RNA aptamer, partial and full-length antibodies, VEGF receptor decoy, and tyrosine kinase inhibitors are being used, and repeated injections are necessary. To shrink abnormal blood vessels in a patient with diabetic retinopathy, scatter or focal laser treatment (photocoagulation) is used, and vitrectomy is performed to remove blood from the vitreous and any scar tissue. These procedures help slow the progression of diabetic retinopathy, but do not provide a long-term solution. Sometimes they fall short of providing a cure, because, by that time, the disease has progressed too far to expect a complete and quick halt in its further progression. There is a strong need to intervene early and aggressively to prevent the progression of these sight-threatening diseases.

**Age-Related Macular Degeneration**

Considering that long-term ROS exposure damages RPE, diets rich in antioxidants have become a good tool for decreasing the progression of AMD to the advanced stage. Clinical studies have shown that the progression of AMD can be slowed by nutritional antioxidants and by cessation of smoking, but the hunt for an effective treatment for the disease, which progresses with age, continues. The Age-Related Eye Disease Study (AREDS), which began in 1992, has documented that people with the intermediate stage of dry AMD have benefited in reducing their risk of progressing to advanced AMD by taking an AREDS antioxidant supplement containing zinc 80 mg, vitamin C 500 mg, vitamin E 400 IU, β-carotene 15 mg, and copper 2 mg. This AREDS formula is now routinely recommended to patients in the United States who meet the high-risk criteria for development of advanced AMD.

Dietary intake of lutein and zeaxanthin pigments increases macular pigment optical density, a predictor of the risk and outcome of AMD, and prevents further progression of the disease in patients with nonadvanced AMD. Although, this short-term clinical trial was very small, the results are promising, and lutein and zeaxanthin have now become a part of the ongoing AREDS II study. Epidemiologic studies have also shown that increased dietary intake of the long-chain polyunsaturated fatty acids reduces the risk of advanced AMD.

Treatment of patients with dry AMD with a mixture of eicosapentaenoic acid (EPA, a precursor of DHA) and DHA slows down the progression of AMD. In addition, supplementation with zinc, an important factor for many antioxidant defense enzymes and nuclear regulatory elements, has been shown to benefit elderly patients with dry AMD. Treatment of female patients with daily folic acid (2.5 mg) and vitamins B6 (50 mg) and B12 (1 mg) has shown reduction in the risk of AMD. This raises the potential for benefits of oral supplementation with omega-3 long-chain polyunsaturated fatty acids, zinc, and folic acid for the treatment of early stages of AMD.

With the knowledge gained about other nutrients since the initiation of AREDS, the ongoing clinical trial of AREDS II is also testing the effect of omega-3 fatty acids and/or lutein and zeaxanthin in risk reduction and also on the rate of progression of AMD. Since these micronutrients function not only as antioxidants, but also as anti-inflammatory and antiangiogenic agents, they are expected to combat different aspects of AMD. However, a very small number of AREDS participants have reported urinary tract problems, possibly associated with the zinc supplementation. Also, due to large doses of β-carotene, yellowing of the skin, and in heavy smokers, an increased risk of lung cancer have been reported. The AREDS II trial, therefore, has the additional goal of assessing whether this modified nutritional supplement with reduced zinc and/or no β-carotene provides benefits in reducing the risk of AMD similar to those of the original AREDS supplement.

Thus, as discussed earlier, nutritional supplements have provided encouraging results in slowing down the progression of AMD. AREDS supplementation has become the major nutritional treatment option. Encouraging results from clinical trials with lutein and zeaxanthin, though small, have encouraged their use in the ongoing AREDS II trial. There is great hope that the outcome of AREDS II will help resolve the question of their benefit.

**Diabetic Retinopathy**

As with AMD, diabetic retinopathy is also a slow-progressing disease. The prevalence of retinopathy is approximately 25% at 5 years of diabetes, but increases to 90% at 20 years. Inhibition of the development of this chronic disease requires a therapy that is safe and easily tolerated. Although cutting-edge research to understand the pathogenesis of diabetic retinopathy is under way in many laboratories with the hope of identifying the molecular targets for therapeutic intervention, the maintenance of good glycemic control remains one of the most effective options for patients with this life-long disease.

As mentioned, oxidative stress is considered one of the major contributors to the development of diabetic retinopathy. The use of antioxidants in animal models of diabetic retinopathy has provided promising results. Prevention studies using two different models of diabetic retinopathy (streptozotocin-induced or experimentally galactosemic rats) have shown that long-term administration of vitamins C and E (10 g/kg and 1 g/kg diet, respectively) ameliorates the development of early signs of retinopathic acellular capillaries and pericyte ghosts in diabetic rats, but not in experimentally galactosemic rats. Vitamin C supplementation decreases the leukostasis and increases iris blood flow perfusion in diabetic rats, suggesting that it has potential in preventing leukostasis, which precedes the development of diabetic retinopathy. However, when vitamins C+E are supplemented with other antioxidants (multi-antioxidants: vitamin C 1 g/kg, trolox 500 mg/kg, α-tocopherol acetate 250 mg/kg, N-acetyl cysteine 200 mg/kg, β-carotene 45 mg/kg, and selenium 0.1 mg/kg of diet), the formation of acellular capillaries and pericyte ghosts is ameliorated in both diabetic rats and experimentally galactosemic rats. This antioxidant mixture also prevents other abnormalities implicated in the development of diabetic retinopathy, including abnormalities in the retinal antioxidant defense mechanism and activation of retinal protein kinase C, nuclear transcriptional factor, and the apoptosis execution enzyme, caspase-3. Furthermore, others have shown that administration of green tea, rich in polyphenolic compounds with potent antioxidant activity, to diabetic rodents improves the histopathology characteristic of diabetic retinopathy. Zeaxanthin supplementation in diabetic rodents significantly inhibits oxidative damage and protects the retina from the inflammatory mediators associated with the pathogenesis of diabetic retinopathy. Lutein decreases the total retinal thickness and loss of ganglion cells and protects impairment in the ERGs. Curcumin, a polyphenol with potent antioxidant and anti-inflammatory effects, decreases oxidative stress and proinflammatory markers in the retina of diabetic rodents. In addition, curcumin significantly decreases histone acetyl transferase activity and cytokine production, possibly via an epigenetic mechanism, and deacetylation of histone is considered important in the development of diabetic retinopathy. These animal studies have raised the possibility that the natural compounds have the potential to inhibit the development of retinopathy in diabetic patients.

We have shown that long-term administration of a diet supplemented with AREDS antioxidants to diabetic rats prevents the retina from oxidative and nitrosative damage. This supplement also protects the integrity of the mitochondrial...
electron transport complex III, and the formation of acellular capillaries, a histopathology marker that is the hallmark of early stages of retinopathy, is significantly decreased. Long-term administration of a stable MnSOD mimetic induces increases in nitrotyrosine levels and attenuates the antioxidant glutathione, ameliorates hyperglycemia-induced apoptosis of retinal capillary cells, and diabetic mice overexpressing MnSOD are protected from the early signs of retinopathy, suggesting possible implications of MnSOD mimetics for diabetic retinopathy. Furthermore, as mentioned earlier, increased peroxynitrite, via DNA single-strand breaking, can activate PARP. Treatment with FP15, a potent peroxynitrite decomposition compound, decreases diabetes-induced increased leukocyte entrapment in the retinal microcirculation and prevents the early lesions of diabetic retinopathy. Thus, it is safe to predict that superoxide dismutase mimetics, peroxynitrite decomposition compounds, and PARP inhibitors would provide attractive options for slowing down the progression of diabetic retinopathy.

Benfotiamine, a fat-soluble synthetic derivative of vitamin B₁, reduces the severity of diabetic retinopathy in rodents, and the mechanism appears to be the inhibition of the major metabolic pathways involved in the pathogenesis of diabetic retinopathy. α-Lipoic acid, which scavenges ROS and regenerates the antioxidant glutathione, ameliorates hyperglycemia-induced increases in nitrotyrosine levels and attenuates mitochondrial DNA damage and the decline of retinal mitochondrial and cytosolic ratio of NAD⁺ to NADH. In animal models of diabetic retinopathy, long-term administration of α-lipoic acid has shown encouraging results. Its administration inhibits diabetes-induced retinal lipid peroxidation and capillary cell apoptosis, accompanied by a decrease in the number of acellular retinal capillaries and pericyte ghost formation. Thus, animal models have suggested that long-term supplementation with antioxidants could be an achievable and inexpensive adjunct therapy to help ameliorate the loss of vision in diabetic patients.

Although animal studies using dietary supplements have provided positive results, clinical trials have been limited, and the results have been inconclusive. In patients with diabetic retinopathy, supplementation with zinc glucoside treatment increases the activity of glutathione peroxidase, an antioxidant defense enzyme with activity that decreases in the retina in diabetes. Calcium dobesilate (2,5-dihydroxybenzensulfonate), a compound with potent antioxidant capacity against hydroxyl radical, and pycnogenol (with both free radical scavenging and anti-inflammatory properties) reduce the progression of diabetic retinopathy. In a small clinical trial, oral administration of a benfotiamine and α-lipoic acid is shown to normalize the major pathways implicated in the development of diabetic complications, including retinopathy. Furthermore, oral vitamin E administration increases retinal blood flow and normalizes hemodynamic abnormalities in the retina of diabetic patients. In contrast, others have found no significant associations between serum levels of major dietary antioxidants and retinopathy. A retrospective study in type II diabetic patients failed to report any association between antioxidant supplementation (vitamins C and E and β-carotene) and decrease in the severity of retinopathy. However, it should be acknowledged that the study was based on a single 24-hour diet recall and was not well controlled. Hence, although intake of vitamin supplements could have some benefit in protecting against retinopathy in diabetic patients, the correlation between retinopathy and dietary antioxidant therapy remains elusive, and the differences in such discrepancies are not clear. Since diabetic retinopathy is a progressive disease, the prior damage determines the outcome of good glycemic control, and early intervention seems to be crucial in preventing its progression. The possibility that these patients started using antioxidants subsequent to the development of early stages of retinopathy, in contrast to the animal studies where antioxidants were administered soon after induction of diabetes, cannot be ruled out. Another possibility is that the antioxidant concentrations in the retina, especially to the mitochondria (the source and the target of ROS) do not reach high enough levels to produce beneficial effects. The limited clinical data, however, pave a path for more comprehensive testing of antioxidants in clinical settings.

VEGF antagonists and corticosteroids are routinely used to treat clinically significant diabetic macular edema. Recent clinical trials using an orally active inhibitor of the β isoform of protein kinase C, Ruboxistaurin, originally designed to inhibit diabetic retinopathy, has produced exciting results, in reducing the progression of macular edema, and preventing the associated decline in visual acuity. However, as with diabetic retinopathy, one of the most viable treatments remains good metabolic control that inhibits the biochemical cascade responsible for its development.

**Is There a Place for Antioxidant Therapy in the Prevention and Treatment of Diabetes-Related Eye Diseases?**

As mentioned earlier, AMD and diabetic retinopathy constitute the major cause of blindness in adults (65 years of age and older) and in working adults (20–70 years). Blindness carries a heavy burden of human and socioeconomic consequences. Since these diseases are slowly progressing chronic diseases, it is important to halt or slow down their progression to prevent vision loss. The overall goal of treatment of a disease is to target the underlying cause of that disease, but despite extensive research in their respective areas, standard therapeutic modalities for AMD and diabetic retinopathy are not available. For AMD patients, AREDS antioxidants have provided promising results, and trials with AREDS II are under way. However, for patients with diabetic retinopathy, because of the possible reasons discussed above, a very limited number of studies with antioxidant supplements have resulted in ambiguous results.

Rodent models of diabetic retinopathy have yielded hopeful results, but retinopathy progresses to only the early stages of the pathology in these models, and as seen in diabetic patients, retinopathy in rodent models does not advance to neovascularization. Another challenge is the identification of the best molecular pathway that can be targeted for the disease. The possibility that antioxidants, administered in combination with the agents targeted for other biochemical abnormalities (including PKC and AGEs) could provide better protection for this complex disease than when used as a monotherapy should be addressed. Moreover, since diabetes-induced metabolic abnormalities apparently are interrelated in the retina, a ther-
apy targeted toward inhibiting one abnormality could have multiple effects on retinal dysmetabolism. Because of a complex etiology of this slow-progressing complication of diabetes, the maintenance of tight glycemic control and the early detection of the disease remain the best available treatment strategies for diabetic retinopathy. Maintenance of such tight glycemic control for chronic disease, however, comes with many challenges, such as the risk of hypoglycemia, lifestyle adjustment by the patient and loved ones, and the costs involved in maintaining such controls. Our novel finding with AREDS antioxidants in rodents is the first step toward testing these antioxidants (or AREDS II) in controlled clinical settings for the treatment of diabetic retinopathy. This trial could be very promising for diabetic patients because the micronutrients that are being used for another chronic ocular disease (AMD), if successful for retinopathy, will allow patients to fight this slow-progressing blinding complication by supplementing their best possible, sensible glycemic control with these nutritional supplements. Thus, we believe that there is a valid place for antioxidant therapy in the prevention and treatment of eye disease.

Acknowledgments

The authors thank Tamer H. Mahmoud and Robert N. Frank, Wayne State University, for helpful suggestions.

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